PATENT
Attorney Docket No. **MYCOLOGX-04875**

COMPLETE LISTING OF CLAIMS
PURSUANT TO WAIVER OF 37 CFR § 1.21

1-11. (Canceled)

12. (Currently amended) A method for ~~the~~ identification of a compound ~~having the ability to inhibit a~~ which inhibits microbial ketol-isomerase activity, comprising the sequential steps of:

a) providing:
   i) a microbial ketol-isomerase,
   ii) fructose-6-phosphate,
   iii) glutamine,
   iv) glutamate dehydrogenase,
   ~~v) nicotinamide adenine dinucleotide,~~
   vi) nitro blue tetrazolium chloride,
   vii) phenazine methosulfate, and
   viii) a candidate compound; ~~and~~

a1

b) preparing a first and a second reaction mixture, wherein said first reaction mixture comprises said microbial ketol-isomerase, said fructose-6-phosphate and said glutamine, and wherein said second reaction mixture comprises said microbial ketol-isomerase, said fructose-6-phosphate, said glutamine and said candidate compound;

c) exposing said first and second reaction mixtures to conditions ~~wherein said microbial ketol-isomerase is capable of producing~~ suitable for production of glucosamine-6-phosphate and glutamate;

d) inactivating said microbial ketol-isomerase in said first and second reaction mixtures;

e) combining said first and second reaction mixtures with said glutamate dehydrogenase, said nicotinamide adenine dinucleotide, said nitro blue tetrazolium chloride, and said phenazine methosulfate under conditions ~~wherein said nitro blue tetrazolium chloride is capable of yielding a~~

suitable for production of a chromogenic product, wherein the quantity of said chromogenic product produced is proportional to activity of said microbial ketol-isomerase ~~activity~~;

f) comparing ketol-isomerase activities in said first and second reaction mixtures, and

R1

g) identifying a candidate compound ~~having the ability to inhibit said~~ which inhibits microbial ketol-isomerase activity, wherein the microbial ketol-isomerase activity of said first reaction mixture is greater than the microbial ketol-isomerase activity of said second reaction mixture.

13. (Original) The method of Claim 12, wherein said microbial ketol-isomerase comprises a crude microbial cell lysate, selected from the group consisting of fungal cell lysates and bacterial cell lysates.

14. (Original) The method of Claim 13, wherein said crude microbial cell lysate is selected from the group consisting of *Aspergillus* cell lysates, *Candida* cell lysates, *Cryptococcus* cell lysates, *Histoplasma* cell lysates, *Pneumocystis* cell lysates, *Rhizopus* cell lysates, *Saccharomyces* cell lysates, *Schizosaccharomyces* cell lysates, *Escherichia* cell lysates, *Staphylococcus* cell lysates and *Pseudomonas* cell lysates.

15. (Original) The method of Claim 12, wherein said inactivating step is selected from the group consisting of boiling and heating to 70°C.

16. (Original) The method of Claim 12, further comprising a clarifying step after the inactivating step, wherein said clarifying step is selected from the group consisting of centrifugation, filtration and a combination thereof.

17. (Currently amended) The method of Claim 12, ~~wherein said compound has the ability to inhibit a microbial ketol-isomerase activity, and~~ further comprising testing said compound which inhibits said microbial ketol-isomerase activity, for antimicrobial activity using a testing means.

R2

18. (Original) The method of Claim 17, wherein said testing means comprises at least one method selected from the group consisting of agar diffusion assays, broth dilution assays, *in vivo* mouse candidosis assays, and *in vivo* mouse aspergillosis assays.

19. (Currently amended) The method of Claim 12, ~~wherein said compound preferentially inhibits said microbial ketol-isomerase compared to a second ketol-isomerase, and wherein said method~~ further ~~comprises~~ comprising the steps of:

h) providing a second ketol-isomerase selected from the group consisting of plant ketol-isomerases and animal ketol-isomerases; ~~and~~

i) preparing a third and a fourth reaction mixture, wherein said third reaction mixture comprises said second ketol-isomerase, said fructose-6-phosphate and said glutamine, and wherein said fourth reaction mixture comprises said second ketol-isomerase, said fructose-6-phosphate, said glutamine ~~and said candidate compound,~~

j) exposing said third and fourth reaction mixtures to conditions ~~wherein said second ketol-isomerase is capable of producing~~ suitable for production of glucosamine-6-phosphate and glutamate,

k) inactivating said second ketol-isomerase in said third and fourth reaction mixtures,

l) combining said third and fourth reaction mixtures with said glutamate dehydrogenase ~~activity~~, said nicotinamide adenine dinucleotide, said nitro blue tetrazolium chloride, and said phenazine methosulfate under conditions ~~wherein said nitro blue tetrazolium chloride is capable of yielding~~ suitable for production of a chromogenic product, wherein ~~production~~ the quantity of said chromogenic product is proportional to the activity of said second ketol-isomerase ~~activity~~,

m) comparing ~~said second~~ ketol-isomerase activities in said third and fourth reaction mixtures, and

n) identifying a compound which preferentially inhibits said microbial ketol-isomerase activity compared to said second ketol-isomerase activity.

20. (Original) The method of Claim 19, wherein said animal ketol-isomerases are mammalian ketol-isomerases.

21. (Original) The method of Claim 20, wherein said mammalian ketol-isomerases are selected from the group consisting of rat ketol-isomerases and human ketol-isomerases.

22. (Original) The method of Claim 19, wherein said second ketol-isomerase comprises a cell lysate.

23. (Original) The method of Claim 19, wherein said second ketol-isomerase is purified.

24. (Original) The method of Claim 19, wherein said second ketol-isomerase is a ~~recombinant ketol-isomerase.~~

25. (Original) The method of Claim 24, wherein said recombinant ketol-isomerase is a recombinant human ketol-isomerase.

26. (Original) The method of Claim 19, wherein said inactivating step is selected from the group consisting of boiling and heating to 70°C.

27. (Original) The method of Claim 19, further comprising a clarifying step after the inactivating step, wherein said clarifying step is selected from the group consisting of centrifugation, filtration and a combination thereof.

28. (Currently amended) The method of Claim 19, ~~wherein said compound preferentially inhibits said microbial ketol-isomerase activity compared to a second ketol-isomerase activity, and~~ further comprising testing said compound which preferentially inhibits said microbial ketol isomerase activity, for antimicrobial activity using a testing means.

AY

29. (Original) The method of Claim 28, wherein said testing means comprises at least one method selected from the group consisting of agar diffusion assays, broth dilution assays, *in vivo* mouse candidosis assays, and *in vivo* mouse aspergillosis assays.

30. (New) A method for identification of a compound which inhibits ketol-isomerase activity, comprising the steps of:

a) providing:
   i) a sample comprising a ketol-isomerase,
   ii) fructose-6-phosphate,
   iii) glutamine,
   iv) glutamate dehydrogenase,
   v) nicotinamide adenine dinucleotide,
   vi) a redox-sensitive chromogenic substrate,
   vii) phenazine methosulfate, and
   viii) a candidate compound;

b) preparing a first and a second reaction mixture, wherein said first reaction mixture comprises said sample, said fructose-6-phosphate and said glutamine, and wherein said second reaction mixture comprises said sample, said fructose-6-phosphate, said glutamine and said candidate compound;

c) exposing said first and second reaction mixtures to conditions suitable for production of glucosamine-6-phosphate and glutamate;

d) inactivating said ketol-isomerase in said first and second reaction mixtures;

e) combining said first and second reaction mixtures with said glutamate dehydrogenase, said nicotinamide adenine dinucleotide, said tetrazolium salt, and said phenazine methosulfate under conditions suitable for production of a chromogenic product, wherein the quantity of said chromogenic product produced is proportional to activity of said ketol-isomerase; and

f) measuring the amount of said chromogenic product is said first and second reaction mixtures.

31. (New) The method of Claim 30, wherein said measuring is accomplished by spectrophotometric analysis.

32. (New) The method of Claim 30, wherein said sample is selected from the group consisting of a fungal sample, a bacterial, sample, an animal sample and a plant sample.

33. (New) The method of Claim 30, wherein said ketol-isomerase is in a form selected from the group consisting of a crude cell lysate, a purified ketol-isomerase and a recombinant ketol-isomerase.

34. (New) The method of Claim 30, wherein said redox-sensitive chromogenic substrate is nitro blue tetrazolium chloride.

AS

35. (New) The method of Claim 30, wherein said redox-sensitive chromogenic substrate is selected from the group consisting of 3-(4,5-dimethyl-2-thiazolyl(-2,5-diphenyl-2H-tetrazolium bromide and sodium 3,3-[(phenylamino)carbonyl]-3,4-tetrazolium-bis(4-methoxy-6-nitro)benzenesulfonic acid hydrate.

36. (New) A kit for identification of a compound which inhibits ketol-isomerase activity, comprising:

a) fructose-6-phosphate,
b) glutamine,
c) glutamate dehydrogenase,
d) nicotinamide adenine dinucleotide,
e) a redox-sensitive chromogenic substrate,
f) phenazine methosulfate, and
g) instructions for testing whether a candidate compound inhibits ketol-isomerase activity.

37. (New) The kit of Claim 35, wherein said redox-sensitive chromogenic substrate is nitro blue tetrazolium chloride.

38. (New) The kit of Claim 35, wherein said redox-sensitive chromogenic substrate is selected from the group consisting of 3-(4,5-dimethyl-2-thiazolyl(-2,5-diphenyl-2H-tetrazolium bromide and sodium 3,3-[(phenylamino)carbonyl]-3,4-tetrazolium-bis(4-methoxy-6-nitro)benzenesulfonic acid hydrate.

a5

39. (New) The kit of Claim 35, further comprising a sample comprising a ketol-isomerase, wherein said sample is selected from the group consisting of a fungal sample, a bacterial, sample, an animal sample and a plant sample.

40. (New) The kit of Claim 39, wherein said ketol-isomerase is in a form selected from the group consisting of a crude cell lysate, a purified ketol-isomerase and a recombinant ketol-isomerase.